



09/886,937

AMENDMENT TO THE SPECIFICATION

Page 11, line 23 (insert Figure 2 description)

cocktail is placed into individual vessels the present invention includes at least a portion of said whisker cocktail being located in a vessel with not less than 3ml of cells pcv.

In yet another embodiment of the invention there is provided an improved whisker mediated method for transforming a plant cell. In this improved method are the steps of contacting at least one cell with a multiplicity of whiskers and with DNA whereby forming a whisker cocktail; and shaking such cocktail in a nonrandom pathway in at least two of the X, y and Z axes wherein said DNA is capable of being inserted into at least one of said cells thus forming a whisker mediated transformed plant cell.

In another one embodiment of the invention there is provided a whisker mediated method for transforming a plant cell: This method comprises the following steps: contacting at least one cell with a multiplicity of whiskers and with DNA whereby forming whisker cocktail; shaking such cocktail for at least 2 seconds wherein said DNA is capable of being inserted into at least one of said cells thus forming a whisker mediated transformed plant cell. Preferably the shaking is not longer then 59 seconds.

Brief description of the Drawings

Figure 1 is a picture of a modified red devil model 5400 paint shaker.

Figure 2 is a graph showing transgenic clone recovery as a function of shaking duration with the paint shaker system. Axis x of the graph represents Shaking Duration and axis Y represents PCR+ Clone Recovery. The material shaken was 36ml P.C.V. of cells, 85 mg of DNA, 5.4 ml of a 5% whisker solution, 14 ml of liquid N6 medium.

Detailed Description of the Invention

Whiskers refers to a genetic engineering technology for plant cells using small bits of silicon carbide or "whiskers". The technology is simple, providing a noncomplex DNA delivery method. The technology employs a small needle-like silicon carbide "whisker". Other material with the needle like shape could be employed. One type of Whiskers is available as Silar SC-9 from Advanced Composite Materials Corp. Greer SC, USA. Whiskers are approximately 0.6 microns in diameter and 10-80 microns in length). Whiskers are used in a cell transformation method in the following manner. A cocktail is composed of DNA, cells and "whiskers" (other components can be added). The DNA can include for example genes, selectable marker gene, introns, promoters

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PAGE 18, line 34 amend as follows:**

2. Osmotically pretreat for 45 minutes at 25°C at 125 r.p.m..
3. Combine the contents (36 ml P.C.V.) from all 3 osmotic flasks into one 250 ml centrifuge bottle and allow the cells to settle well. Draw off 190 to 200 ml liquid and save for re-use.
4. Add 8.1 to 10.8 ml of a 5% whisker solution (5% w/v in liquid N6 Osmotic medium) and 170 ul of plasmid DNA to the centrifuge bottle + cells, and agitate on the paint shaker for the desired length of time (5-20 seconds).
5. Transfer all contents from the centrifuge bottle to the flask containing the N6 osmotic that was drawn off earlier.
6. Add 125-130 ml fresh N6 liquid (final total volume should be 375 ml) for a 2- 20 hour post whiskering recovery period on a platform shaker (25°C, 125 r.p.m.).
7. Isolate 15 ml of processed tissue suspension onto a filter paper (110 mm) using a poly Buchner funnel apparatus. Approximately 25 filters of tissue are derived from each bottle of whiskered tissue

The filter was placed on an N6 solid medium plate with sterile forceps and the plates wrapped in 3M micropore tape. The platees were then incubated at 28° C in the dark and examined for clones at 4,6,8 weeks after embedding.

Commercially competent cell lines must be able to produce adequate numbers of regenerable transgenic clones that ultimately result in the production of fertile transgenic plants. For example when 35 ml of PVC cells are employed an adequate numbers of clones for one whisker mediated transformation with the shaking movement of the present invention is 10 PCR positive clones, and more preferably 20 and yet more preferably 24 and yet more preferable 40 and above (see figure 242).

AMENDMENT TO THE SPECIFICATION

PAGE 19, lines 3-17 amend as follows:

DELETE CHART



CERTIFICATE OF MAILING UNDER 37 C.F.R. 1.8

I hereby certify that the foregoing Amendment after Final, Remarks, Certificate of Mailing, Amendments to the Specification, new sheet, Figure 2, and postcard are being mailed to Commissioner for Patents, PO Box 1450, Alexandria, VA 22313-1450, on this 24 day of May, 2007.

A handwritten signature that appears to read "Dennis Worcester".